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Exploiting the enantioselectivity of Baeyer-Villiger monooxygenases via boron oxidation

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ABSTRACT

The enantioselective carbon–boron bond oxidation of several chiral boron-containing compounds by Baeyer-Villiger monooxygenases was evaluated. PAMO and M446G PAMO conveniently oxidized 1-phenylethyl boronate into the corresponding 1-(phenyl)ethanol (ee = 82–91%). Cyclopropyl boronic esters were also oxidized but with no enantioselectivity. β-Boryl carboxylic esters were not oxidized by any BVMOs.

1. Introduction

Baeyer-Villiger monooxygenases (BVMOs) are flavin-containing and NAD(P)H-dependent enzymes that catalyze the incorporation of one oxygen atom into organic substrates.[1] BVMOs have recently been shown to be excellent alternatives for performing the oxidation of aldehydes and ketones to their corresponding esters, the oxygenation of heteroatoms (sulfur, nitrogen, phosphorus, boron, and selenium) and even epoxidation reactions.[2] In many of these reactions, the transformation of the substrates occurs with high enantio- and/or regioselectivity, using environmentally friendly conditions.[3] Cyclohexanone monooxygenase (CHMO), which is one of the most studied BVMOs, has a broad substrate acceptance and displays moderate thermostability.[4] In contrast, phenylacetone monooxygenase (PAMO) is remarkably stable but is limited in substrate range by its preference for aromatic compounds. From this BVMO, a mutant that has an altered substrate acceptance profile has been generated (M446G PAMO), and accepts a different range of aromatic ketones and aldehydes while retaining thermostability. 4-Hydroxyacetophenone monooxygenase (HAPMO)[3d,5,6] is another BVMO that has been shown to be effective in converting aromatic ketones.

Recently, we described the first example of the kinetic resolution of boron-containing chiral compounds mediated by PAMO.[7] We have been studying boron-containing compounds because they are known to be versatile intermediates in organic synthesis. The C–B bond can be easily oxidized leading to the release of boron and the formation of a C–O bond, resulting in alcohols, aldehydes, or ketones, among other reactions.[8] Additionally, chiral organoboron compounds are especially important in organic synthesis, since they can be converted into other functional groups with retention of configuration and used as chiral catalysts.[9]

Based on previous reports of the selectivity of BVMO and the importance of boron compounds in organic synthesis, herein we explore the enantioselectivity of BVMOs for oxidative kinetic resolutions of chiral organoboron compounds (Fig. 1).

2. Results and discussion

2.1. Synthesis of boron substrates for the evaluation of the enantioselectivity of BVMOs

The set of chiral boron substrates studied is shown in Figure 1. The organoboron compounds were synthesized according to Figure 2.

2.2. Evaluation of the enantioselectivity of BVMOs via boron oxidation

Motivated by our previous result, in which 1-phenylethyl boronate **1a** was submitted to oxidative kinetic resolution mediated by PAMO (Table 1, entry 1),[8] we performed the kinetic resolution of **1a** and other boron-containing aromatic compounds **1b** and **1c** by using PAMO, M446G PAMO, CHMO, and HAPMO (Table 1). In these reactions we used a cofactor regenerating system by applying phosphite dehydrogenase (PTDH) and a sacrificial substrate (phosphite).

We observed that in all cases CHMO and HAPMO led to the decomposition of the starting material with no desired product being observed. PAMO only mediated the kinetic resolution of **1a** ($E = 33$, Table 1, entry 1). Conversely, M446G PAMO mediated the oxidation reaction of **1a** (1-phenylethyl boronate, $E = 9.9$) and **1b** (4-methoxy-1-phenylethyl boronate, $E = 2.8$), but no conversion of **1c** was observed (4-fluoro-1-phenylethyl boronate) (Table 1,

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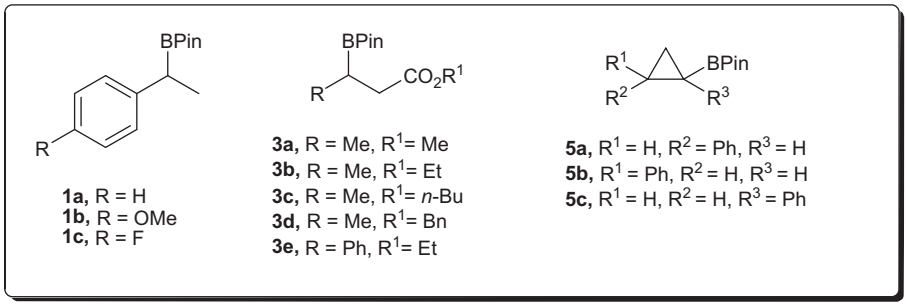


Figure 1. Boron-containing substrates used for the evaluation of the enantioselectivity of BVMOs in an oxidative kinetic resolution process.

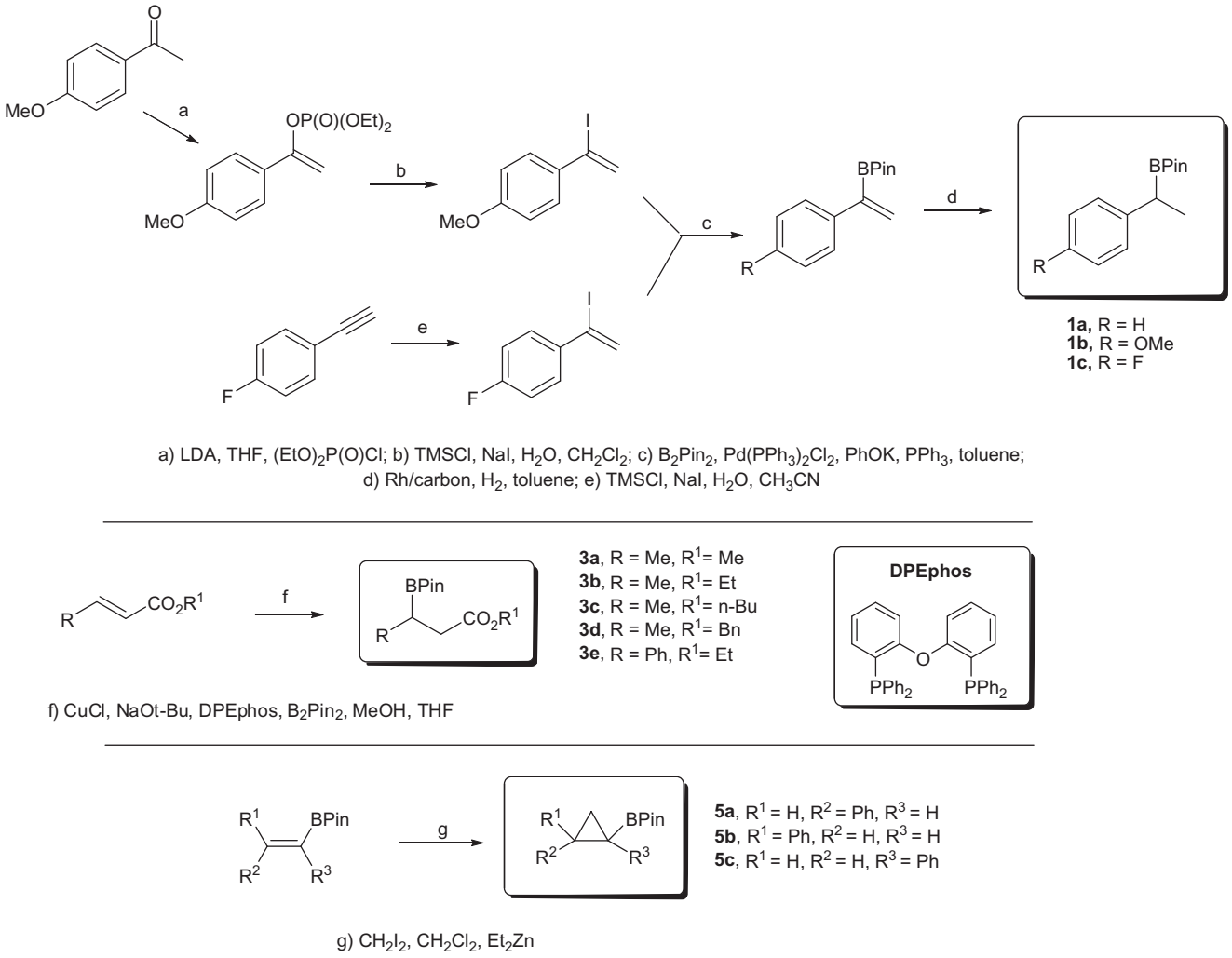


Figure 2. Synthesis of boron substrates.

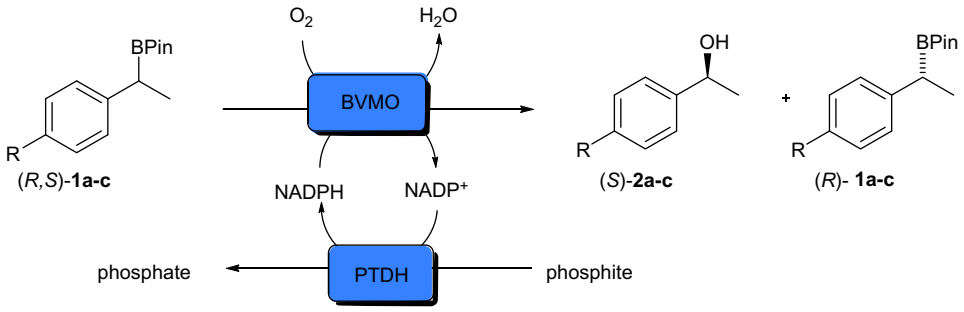
entries 2, 4, and 6). It has been reported that the oxidation of the C–B bond mediated by a monooxygenase occurs with retention of configuration, similar to non-enzymatic oxidations, resulting in the formation of the alcohol derivatives.[10] PAMO and M446G PAMO reacted both with the (*S*)-enantiomer leading to the (*S*)-alcohol and the (*R*)-boronic ester.

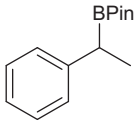
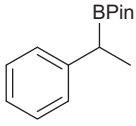
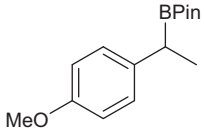
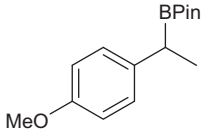
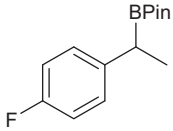
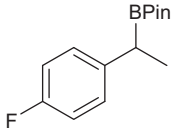
We also explored the oxidation reaction of β-boronated carboxylic esters to give β-hydroxy carboxylic esters. β-Hydroxy carboxylic esters are highly attractive synthetic targets due to their versatility in organic chemistry. Additionally, optically active β-hydroxy carboxylic acids are important and interesting building blocks for the synthesis of biologically active compounds.[11]

The results obtained with PAMO (Table 1) led us to evaluate the kinetic resolution of β-boronated carboxylic esters **3a**–**e** mediated by this monooxygenase (Scheme 1).

Unfortunately, compounds **3a**–**b** were not stable in the Tris-buffer, and even with no enzyme in the reaction medium, we observed a decomposition of starting material. On the other hand, compounds **3c**–**e** were stable under the reaction conditions but again no reaction was observed.

Table 1
Evaluation of the enantioselectivity of the BVMO-catalyzed oxidation of phenylethyl boronates **1a–c**[a]



Entry	Substrate	Enzyme (BVMO)	Time (h)	Conv. (%)	ee (*S*)-**2a–c** (%)	ee (*R*)-**1a–c** (%)
1	 **1a**	PAMO	5	49	91	85[8]
2	 **1a**	M446G PAMO	4	50	82	70
3	 **1b**	PAMO	24	(−)[b]	(−)[c]	(−)[c]
4	 **1b**	M446G PAMO	7	52	50	47
5	 **1c**	PAMO	24	(−)[b]	(−)[c]	(−)[c]
6	 **1c**	M446G PAMO	24	(−)[b]	(−)[c]	(−)[c]

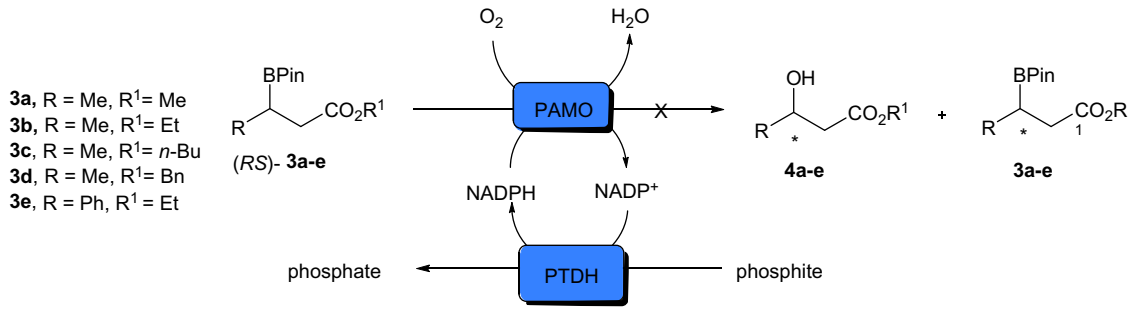
[a] Tris buffer 50 mM pH 7.5, DMSO (1%), substrate (10 mM), phosphite (20 mM), PTDH (1 μM), NADPH (0.2 mM), and BVMO (4 μM).
[b] No reaction was observed.
[c] No enantiomeric excess was observed.

We also carried out the kinetic resolution of cyclopropyl boronic esters **5a–c** (Table 2). In addition to the importance of boron-containing substrates, cyclopropyl compounds are versatile building blocks in the synthesis of natural products due to their well-defined three-dimensional structures.[12]

We used three cyclopropyl derivatives with different geometries. CHMO was unable to perform the oxidation of any of these compounds **5a–c**. Conversely, PAMO, M446G PAMO, and HAPMO catalyzed the reaction, but with no enantioselectivity regardless of geometry. Even with the phenyl moiety close to the boronic ester moiety **5c**, no enantioselectivity was observed.

3. Conclusion

We have shown that PAMO was able to oxidize aromatic boron compounds with no substituent in the aromatic ring with great enantioselectivity. It could also be used to convert cyclopropyl derivatives but with no enantioselectivity. PAMO was unable to oxidize 1-phenylethyl boronates with substituents on the aromatic ring (4-OMe and 4-F) apart from β-borylated carboxylic esters. The mutant M446G PAMO showed a similar substrate acceptance, but this BVMO displayed a lower enantioselectivity. We observed that HAPMO can catalyze cyclopropyl boronic ester oxidations, but with



Scheme 1. Attempts to promote the oxidative kinetic resolution of β-boronated carboxylic esters **3a–e** mediated by PAMO.

no enantioselectivity. CHMO was unable to oxidize any of the tested substrates. We can conclude that the substituent on the aromatic ring of 1-phenylethyl boronates affects the performance of PAMO and M446G PAMO. Moreover, cyclopropyl boronic esters were efficiently oxidized to cyclopropyl alcohols by PAMO, M446G PAMO, and HAPMO, but no enantioselectivity was observed.

4. Experimental

4.1. General

Recombinant phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*, M446G PAMO mutant, 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* and cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. were overexpressed and purified as previously described.[13] Phosphite dehydrogenase was obtained as described before.[14] Phosphite and NADPH were purchased from Sigma–Aldrich.

High performance liquid chromatography (HPLC) analyses for measurement of enantiomeric excesses were performed on a Shimadzu (diode array detector SPD-M1OA, auto injector SIL-10AD e Column Oven CTO-10A).

4.2. Synthesis of 4,4,5,5-tetramethyl-2-(1-phenylethyl)-1,3,2-dioxaborolane compound 1a[15]

This compound was synthesized according to the literature.[15] Clear oil, 80%. ^{1}H NMR (200 MHz, $CDCl_3$):δ 1.19–1.35 (m, 15H), 2.36–2.46 (m, 1H), 7.12–7.26 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.99, 24.54, 24.58, 83.26, 125.02, 127.74, 128.24, 144.92. LRMS (EI) *m/z* (%) = 232 (M^+, 60), 217(28), 174(14), 146(22), 132(74), 117(30), 105(100), 91(14), 83(64), 77(15), 59(10). FT-IV (film, cm^{-1}) ν_{max}: 3065, 3029, 2981, 1459, 1351, 1146.

4.3. Synthesis of 2-[1-(4-methoxyphenyl)ethyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 1b[16]

The intermediate diethyl 1-(4-methoxyphenyl)vinyl phosphate was synthesized according to the literature.[16a] Orange oil, 75%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.34 (t, *J* = 7.2 Hz, 6H), 3.82 (s, 3H), 4.07–4.20 (m, 4H), 5.10–5.17 (m, 2 H), 6.88 (d, *J* = 9 Hz, 2 H), 7.52 (d, *J* = 9 Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.04, 25.47, 55.18, 64.28, 64.40, 95.36, 113.56, 122.27, 126.50, 152.05, 160.11. The intermediate iodide was synthesized according to the literature[16d] and used without purification in the coupling reaction, which was carried out according to the literature[16e,f] to give 2-[1-(4-methoxyphenyl)vinyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane. Clear oil, 83%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (s, 12 H), 3.77 (s, 3H), 5.94 (d, *J* = 2.8 Hz, 1H), 5.99 (d, *J* = 2.6 Hz, 1H), 6.8 (d, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.77, 55.20, 83.69, 112.08, 113.56, 128.21, 158.80. The reduction of the vinyl boronic ester was carried out in a similar way to procedures described in section 4.2 to give 2-[1-(4-methoxyphenyl)ethyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **1b.** Yellow oil,90%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.12 (s, 12H), 1.24 (s, 3H), 2.28 (q, *J* = 7.8 Hz, 1H), 3.70 (s, 3 H), 6.70–7.14 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 17.33, 24.57, 55.16, 83.18, 113.74, 128.58, 136.99, 157.22. LRMS (EI) *m/z* (%) = 286 (M^+, 4), 206 (5), 178 (4), 151(3), 136 (18), 135 (100), 134 (20), 121 (12), 119 (12), 105 (9), 103 (8), 91 (20), 77 (16), 65 (8). FT-IV (film, cm^{-1}) ν_{max}: 3004, 2919, 1661, 1437, 1407, 1316, 1021.

4.4. Synthesis of 2-[1-(4-fluorophenyl)ethyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 1c[8,17]

The intermediate iodide was synthesized according to the literature[16a] and used without purification in the coupling reaction, which was carried out according to the literature[16e,f] to give 2-[1-(4-fluorophenyl)vinyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane. Yellow solid, 60%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (s, 12H), 6.88–6.98 (m, 2H), 7.36 (d, *J* = 5.4 Hz, 2H), 7.40 (d, *J* = 5.6 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.86, 83.95, 114.79, 115.22, 129.35, 129.53, 129.63, 159.05 (d, *J* = 240.5 Hz). The reduction of the vinyl boronic ester was carried out in a similar way to procedures described in section 4.2 to give 2-[1-(4-fluorophenyl)ethyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **1c**. Yellow oil, 87%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (s, 12H), 1.30 (s, 3 H), 1.61–1.81 (m, 1H), 6.94–7.47 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.13, 24.79, 83.88, 112.11, 114.72, 128.62, 130.51, 156.51 (d, *J* = 233.5 Hz). LRMS (EI) *m/z* (%) = 248 (M^+, 54), 247 (27), 232 (26), 205 (30), 204 (31), 191 (36), 174 (25), 162 (45), 149 (44), 148 (100), 147 (90), 123 (35), 77 (34), 58 (31), 50 (18). FT-IV (film, cm^{-1}) ν_{max}: 2957, 2918, 2850, 1736, 1635, 1380, 1233, 1181, 835.

4.5. Synthesis of β-borylated carboxylic esters 3a–e

These compounds were synthesized according to the literature[11] and the spectroscopic data are in the literature as well.[18]

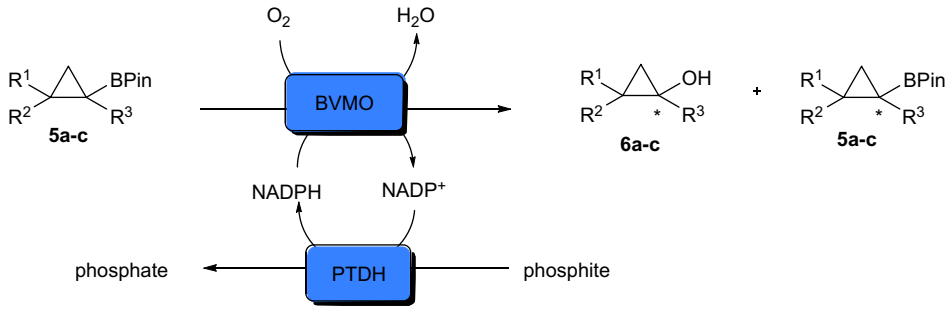
4.6. Synthesis of cyclopropyl boronic esters 5a–c[19]

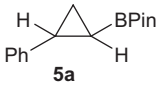
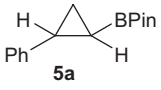
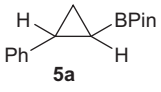
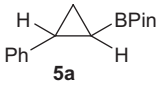
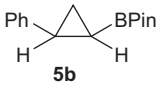
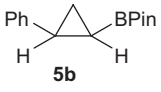
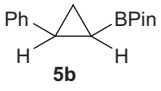
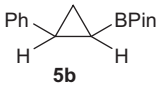
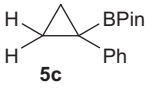
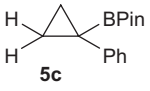
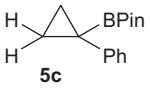
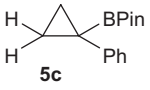
These compounds were synthesized according to the literature.[19]

4.6.1. 4,4,5,5-Tetramethyl-2-(2-phenylcyclopropyl)-1,3,2-dioxaborolane 5a

Clear oil, 82%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.25–0.33 (m, 1H), 0.86–1.03 (m, 2H), 1.29 (s, 12H), 2.04–2.10 (m, 1H), 7.03–7.22 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.05, 21.86, 24.78, 83.31, 125.57, 127.01, 128.19, 128.52, 143.31. LRMS (EI) *m/z* (%): 245 (13), 244 (M^+, 80), 243 (17), 186 (16), 145 (100), 144 (49), 143 (45), 126 (50), 117 (90), 116 (55), 115 (52), 101 (30), 91 (29), 85

Table 2
Evaluation of the enantioselectivity of the BVMO-catalyzed oxidation of cyclopropyl boronic esters **5a–c** oxidation[a]



Entry	Substrate	Enzyme	Time (h)	Conv. (%)[c]
1	 **5a**	PAMO	24	45
2	 **5a**	M446G	16	49
3	 **5a**	HAPMO	24	50
4	 **5a**	CHMO	24	(–)[b]
5	 **5b**	PAMO	24	51
6	 **5b**	M446G	16	46
7	 **5b**	HAPMO	24	55
8	 **5b**	CHMO	24	(–)[b]
9	 **5c**	PAMO	24	50
10	 **5c**	M446G	16	51
11	 **5c**	HAPMO	24	56
12	 **5c**	CHMO	24	(–)[b]

[a] Tris buffer 50 mM pH 7.5, DMSO (1%), substrate (10 mM), phosphite (20 mM), PTDH (1 μM), NADPH (0.2 mM), and BVMO (4 μM).
[b] No reaction was observed.
[c] No enantiomeric excess was observed.

(25), 83 (27). FT-IV (film, cm^{-1}) ν_{max}: 3081, 3061, 3026, 2978, 2930, 1624, 1449, 1419, 1353, 1323, 1212, 1144, 969, 852, 750, 697.

4.6.2. 4,4,5,5-Tetramethyl-2-(2-phenylcyclopropyl)-1,3,2-dioxaborolane 5b

Clear oil, 84%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.28–0.41 (m, 1H), 0.78 (s, 6 H), 0.91 (s, 6H), 0.95–1.06 (m 1H), 1.14–1.22 (m, 2H), 7.00–7.20 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 1.02, 8.86, 21.72, 24.35, 24.74, 82.88, 125.67, 127.62, 128.76, 140.70. LRMS (EI) *m/z* (%): 245 (15), 244 (M^+, 95), 243 (20), 229 (13), 186 (20), 185 (18), 145 (100), 144 (45), 143 (40), 128 (30), 126 (45), 117 (87), 116 (70), 115 (65), 101 (30), 91 (28), 83(25). FT-IV (film, cm^{-1}) ν_{max}: 3083, 3060, 3026, 2978, 2927,1603, 1409, 1322, 1223, 1144, 1028, 859, 699.

4.6.3. 4,4,5,5-Tetramethyl-2-(1-phenylcyclopropyl)-1,3,2-dioxaborolane 5c

Clear oil, 75%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87–0.92 (m, 2H), 1.07–1.12 (m, 2H), 1.20 (s, 12H), 7.2–7.51 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ −4.02, 13.31, 24.57, 83.29, 125.19, 127.14, 127.93, 128.89, 144.79. LRMS (EI) *m/z* (%): 245 (15), 244 (M^+, 90), 243 (17), 229 (16), 187 (55), 171(18), 145 (97), 144 (77), 143 (75), 129 (29), 117 (100), 116 (65), 115 (59), 105 (50), 101 (60), 85 (42), 83 (44). FT-IV (film, cm^{-1}) ν_{max}: 3081, 3059, 2978, 2932, 2867, 1685, 1391, 1318, 1145, 849, 699.

4.7. General procedure for enzymatic oxidation reactions

To a flask (2 mL, Eppendorf®) containing a solution of the starting material (1 M in DMSO, 5 μL) were added Tris/HCl buffer at pH 7.5 (50 mM, 440 μL), phosphite solution (500 mM, 20 μL), NADPH (100 mM, 10 μL), PTDH (100 μM, 5 μL), and enzyme (BVMO) (100 μM, 20 μL). Reactions were shaken at 200 rpm and 30 °C (PAMO and M446G PAMO mutant) or 150 rpm and 25 °C (HAPMO and CHMO) for the time established. The reactions were stopped, extracted with EtOAc (3 × 0.5 mL), dried over Na_2SO_4, and analyzed by chiral HPLC. Control experiments in the absence of an enzyme were performed for all substrates tested, and no reaction was observed.

4.8. General procedure for chemical oxidation of boronic esters 1a–c

To a flask (2 mL, Eppendorf®) containing enantiopure boronic ester **1a–c** (0.1 mmol) were added a 2 M NaOH solution (1 mmol) and H_2O_2 30% (3 mmol). The reaction was shaken for 3 h at room temperature. After this period, the reaction was extracted with EtOAc (3 × 0.5 mL), dried over Na_2SO_4, and analyzed by chiral HPLC.

4.9. Absolute configuration

The absolute configuration of chiral alcohols **2a–c** was established by comparing HPLC chromatograms with the patterns described in the literature.[20] The chiral boron compounds **1a–c** were transformed into the corresponding alcohols **2a–c** (see Section 4.8) to determine their absolute configurations. The absolute configuration of compounds **5a–c** could not be determined because the bio-oxidations were not enantioselective.

4.10. Determination of the enantiomeric excess (ee)

The enantiomeric excesses of compounds **2a–c** and **5a–c** were measured by chiral HPLC analysis. Compounds **1a–c** were transformed into the compounds **2a–c,** and then the ee was collected (see Section 4.8).

The analysis of **2a** was carried out on Column Chiracel® OD, *n*-heptane/*iso*-propanol 95:5, flow rate: 1 mL/min, UV detection: 254 nm, retention times: t_R = 5.00 min (*R*) and t_R = 5.92 min (*S*).

The analysis of **2b** was carried out on Column Chiracel® OD-H, *n*-heptane/*iso*-propanol 97:3, flow rate: 0.8 mL/min, UV detection: 227 nm, retention times: t_R = 40,54 (*S*) and 41,93 (*R*).

The analysis of **2c** was carried out on Column Chiralpak® AS-H, *n*-heptane/*iso*-propanol 98.5:1.5, flow rate: 0.5 mL/min, UV detection: 254 nm, retention time: t_R = 20,11 (*S*) and 21,51 (*R*).

The analysis of **5a–c** was carried out on Column Chiralpak® AD-H, *n*-heptane, flow rate: 0.25 mL/min, UV detection: 220 nm. Retention time of **5a**: t_R = 20.67 and 22.09; retention time of **5b**: t_R = 19.78 and 20.81; retention time of **5c**: t_R = 17.55 and 19.77.

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